Unraveling the catalytic mechanism of the bacteriophage T7 gene 4 helicase

Crampton, D.J.; Mukherjee, Sourav; Oijen, A. van; Richardson, C.C.

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such as switch 1 movement, rather than influencing the switch 2 equilibrium. Consequently, despite actin does not have major effect on nucleotide binding and the equilibriums of the pre- and post-power-stroke the fluxes of the kinetic routes change fundamentally by actin binding: in the absence of actin phosphate release precedes the closed-open transition while in ternary complex the main route is that products releases follow the power stroke.

F1-006
Calmodulin and calmodulin-like protein as light chains for myosin-10: Specificity and role in protein stabilization leading to prolonged function

E. E. Strehler, A. J. Caride, A. S. Mauer and R. D. Bennett
Biochemistry and Molecular Biology, Mayo Clinic College of Medicine, Rochester, MN, United States of America.
E-mail: strehler.emanuel@mayo.edu

Vertebrate myosin-10 (MYO10) is involved in filopodial motility, phagocytosis, and microtubule-F-actin interactions. Each MYO10 heavy chain contains three IQ motifs, which are light chain binding sites. Previous work has shown that the first two IQ domains of MYO10 bind calmodulin (CaM) whereas IQ3 alternatively binds calmodulin-like protein (CLP). We performed stopped-flow experiments with fluorescent derivatives of CaM (TA-CaM) and CLP (TA-CLP) to analyze their binding to the IQ3 peptide. TA-CaM bound to IQ3 in the absence and in the presence of 100 μM Ca^{2+}. In the absence of Ca^{2+} the binding was faster, albeit with lower affinity, suggesting an even faster dissociation rate. In the presence of Ca^{2+}, the time course of the TA-CaM-IQ3 reaction was best described by an exponential function. CLP was able to reduce the amplitude of this exponential in the presence, but not in the absence, of Ca^{2+}. These results suggest a novel IQ domain interaction, in which Ca^{2+} regulates binding of CaM to IQ3 by modulating competition with CLP. In HeLa cells over-expressing CLP, endogenous MYO10 was strongly upregulated. Likewise, transfection with GFP-MYO10 resulted in increased fluorescence in cells that co-expressed CLP or excess CaM. MYO10 upregulation resulted in an increase in the size and number of filopodia. Cells expressing CLP displayed increased motility as indicated by their shortened wound-healing time compared to control cells lacking CLP. CLP-dependent upregulation of MYO10 was due to increased protein stability. CLP expression in specific epithelial cells may thus prolong MYO10 function in conditions of elevated intracellular Ca^{2+} and limiting CaM. Acknowledgment: Supported by grants from the Susan G. Komen Breast Cancer Foundation (EES) and the American Heart Association (AJC).

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Crystal structure of the portal protein from bacteriophage SPP1 and model for DNA translocation

A. A. Lebedev[1], M. H. Krause[2], A. Vagin[1], E. V. Orlova[3], E. J. Dodson[1], P. Tavares[4] and **A. A. Antson**[1]
[1]YSBL, Chemistry Department, York University, York, United Kingdom, [2]Max-Planck Institut für Molekulare Genetik, Berlin, Germany, [3]Birkbeck College, Department of Crystallography, University of London, London, United Kingdom, [4]Unité de Virologie Moléculaire et Structurale, Gif-sur-Yvette, France.
E-mail: fred@ysbl.york.ac.uk

The mechanism of DNA translocation into a viral procapsid remains one of the most intriguing questions of viral particle assembly [1]. Tailed bacteriophages and herpes viruses have a specialized vertex for double-stranded DNA (dsDNA) entry into the procapsid during viral chromosome packaging [2, 3]. The main component of this specific doorway is the portal protein, a circular oligomer with a central tunnel through which the DNA transfer occurs. Together with viral ATPase (terminase) the portal protein forms a molecular motor that is able to translocate DNA against high internal pressure [4]. We determined the X-ray structure of the SPP1 portal protein (gp6) in its 13-subunit oligomeric form where the tunnel residue segments, not visible in the previously determined structures of bacteriophage phi-29 portal protein [5, 6], are well defined and form a DNA-transfer arm. Our X-ray and electron microscopy data suggest that DNA translocation is driven by a novel mechanism involving mechanical movements of arms along the inner walls of the tunnel. We propose a model for DNA translocation where such movements propagate around the double helix of DNA similar to a "Mexican wave" moving across a stadium.

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Unraveling the catalytic mechanism of the bacteriophage T7 gene 4 helicase

D. J. Crampton, S. Mukherjee, A. van Oijen and C. C. Richardson
Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA, USA.
E-mail: dcrampton@hms.harvard.edu

The gene 4 helicase of bacteriophage T7 is a molecular motor that unwinds double-stranded DNA using the energy derived from the hydrolysis of deoxynucleoside 5-triphosphates. Here we present data pertaining to four essential activities of the helicase that act cooperatively to unwind DNA. (i) *Oligomer Formation*. The T7 gene 4 helicase forms both heptamers and hexamers dependent upon the presence of nucleoside di- or triphosphates respectively. We find that the heptamer cannot bind DNA but rather, in the presence of single-stranded DNA, heptamer converts to hexamer when both nucleoside di- and triphosphates are present together. This conversion between oligomers is regulated by histidine 465 through differentiation of the absence or presence of a γ-phosphate on the bound nucleotide. (ii) *DNA Binding*. The loop comprised of residues 466–475 has been postulated to be the major site for the binding of single-stranded DNA. Lysines 471 and 473 of this loop were altered to determine their contribution to the overall binding of single-stranded DNA. (iii) *dTTP Hydrolysis*. We find that all subunits of the hexamer are active in the hydrolysis of dTTP. Changing the catalytic base glutamate 343 to glutamine creates a non-catalytic subunit that responds to dTTP in a manner similar to the effect of non-hydrolyzable analog β,γγ-methylene dTTP on wild-type helicase. The presence of a single non-catalytic subunit per hexameric unit abolishes all dTTPase activity. (iv) *Translocation*. We are developing methods of observing movement along DNA by the gene 4 helicase using single-molecule fluorescence microscopy.